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Cellulose and wheat straw degradation by *Ruminococcus albus* was monitored using NMR spectroscopy. *In situ* solid-state ^{13}C-cross-polarization magic angle spinning NMR was used to monitor the modification of the composition and structure of cellulose and ^{13}C-enriched wheat straw during the growth of the bacterium on these substrates. In cellulose, amorphous regions were not preferentially degraded relative to crystalline areas by *R. albus*. Cellulose and hemicelluloses were also degraded at the same rate in wheat straw. Liquid state two-dimensional NMR experiments were used to analyse in detail the sugars released in the culture medium, and the integration of NMR signals enabled their quantification at various times of culture. The results showed glucose and cellodextrin accumulation in the medium of cellulose cultures; the cellodextrins were mainly cellotriose and accumulated to up to 2 mM after 4 days. In the wheat straw cultures, xylose was the main soluble sugar detected (1.4 mM); arabinose and glucose were also found, together with some oligosaccharides liberated from hemicellulose hydrolysis, but to a much lesser extent. No cellodextrins were detected. The results indicate that this strain of *R. albus* is unable to use glucose, xylose and arabinose for growth, but utilizes efficiently xylooligosaccharides. *R. albus* 20 appears to be less efficient than *Fibrobacter succinogenes* S85 for the degradation of wheat straw.

Ruminococcus albus is a Gram-positive rumen bacterium widely recognized for its high cellulolytic activity. It is the predominant cellulolytic bacterial species found in the rumen of cows [1], but is outnumbered by the other rumen cellulolytic species, *R. flavefaciens* and *Fibrobacter succinogenes*, in the rumen of sheep [2]. *In vitro* studies have shown that *R. albus* becomes predominant over the other two fibrolytic species in co-cultures on cellulose [3,4]. Data have also shown the negative interaction of *R. albus* and *F. succinogenes* on lucerne cell-wall polysaccharide degradation, as well as the complementary effect of *R. albus* and *R. flavefaciens* in lucerne hemicellulose degradation [5]. The interactions of cellulolytic species in fibre degradation therefore appear to be very complex, depending on several factors. An understanding of how the cellulolytic system of each species operates on natural substrates should aid in the determination of these complex interactions. The fibrolytic system of *R. albus* is composed of many different cellulases, xylanases

Abbreviations
^{13}C-CP MAS, ^{13}C-cross-polarization magic angle spinning; HSQC, heteronuclear single quantum coherence; PE, polyethylene; PP, polypropylene; TSP-d_4, sodium 3-(trimethylsilyl) propionate.

and esterases [6]. Although many of these enzymes have been characterized, little is known about their concurrent mode of action on solid substrates.

In the present work, the degradation and metabolism of cellulose and wheat straw by *R. albus* 20 cells growing on these substrates were studied using NMR. A kinetic analysis of the polysaccharides degraded and of the sugars solubilized should aid in the understanding of the action of the cellulolytic system and in the evaluation of its efficiency in the degradation process. We used a combined approach previously developed to examine the action of the *F. succinogenes* S85 fibrolytic system on lignocellulosic fibres [7]. *In situ* solid-state ^{13}C-cross-polarization magic angle spinning (^{13}C-CP MAS) NMR was used to monitor the degradation of cellulose and ^{13}C-enriched wheat straw. The advantages of this method are that: (1) it is nondestructive for the materials being investigated; (2) it resolves as many separate components as possible; and (3) it is quantitative for these components and is straightforward to implement (although a long acquisition time may be necessary). In parallel, liquid state two-dimensional NMR experiments were used to analyse in detail the various sugars released. We also compared the action of *R. albus* and *F. succinogenes* on the solid fibrous substrates.

Results

Growth of *R. albus* on cellulose and wheat straw

R. albus 20 was grown for up to 4 days with 100 mg of cellulose (Sigmacell 20) or ^{13}C-labelled or unlabelled wheat straw. Growth was monitored by the quantification of fermentation products performed by both ^{1}H NMR (Figs 1 and 2) and enzymatic methods. Figure 1 shows an example of the spectra obtained before and after 4 days of culture of *R. albus* 20 on wheat straw. The metabolites acetate (a), lactate (l) and formate (f) were detected and quantified after subtracting the peak areas obtained at $t = 0$ (caused by acids present in the culture medium). Ethanol was quantified on the spectra registered without lyophilization of the extracellular medium. Figure 2 shows that the concentration of these metabolites reached a maximum between 24 and 48 h, and then remained fairly constant. The metabolites were not produced at the same concentration when the cells were grown on cellulose (Fig. 2A) and straw (Fig. 2B), except for lactate. Ethanol and formate concentrations were twofold lower on straw, and acetate was also produced at a slightly lower concentration on this substrate. The metabolite concentrations measured in cellulose cultures were similar to

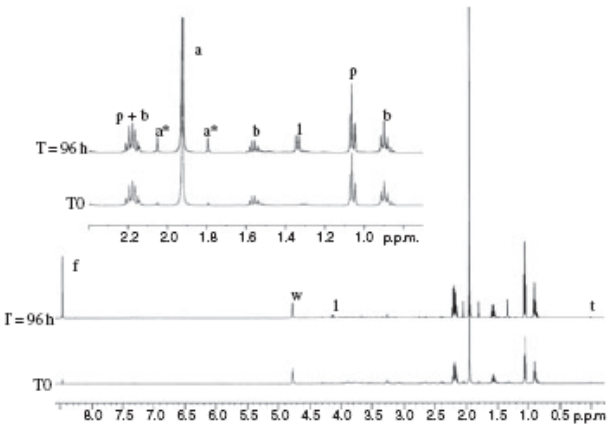

Fig. 1. ^{1}H NMR spectra registered before (T0) and after (T = 96 h) 4 days of growth of *R. albus* 20 on wheat straw. a, acetate; a*, acetate satellites J_{1H-13C}; b, butyrate; f, formate; l, lactate; p, propionate; t, TSP-d_4; w, HOD.

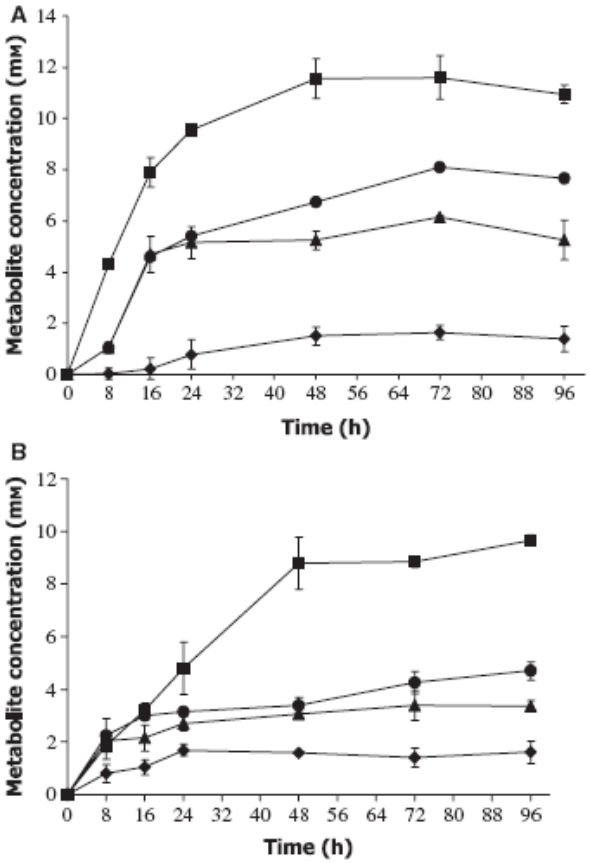

Fig. 2. Metabolites produced during the growth of *R. albus* 20 on cellulose and wheat straw. *R. albus* 20 cells were grown at 38 °C on 10 mL of mineral medium with 100 mg of cellulose (A) or wheat straw (B). Time dependence of acetate (■), formate (▲), lactate (◆) and ethanol (●) concentrations, quantified by ^{1}H NMR. Values are the means ± standard deviation of two experiments.

those found when cellobiose was used as a substrate (not shown). No growth of *R. albus* 20 was obtained when xylose, arabinose or glucose (3 $g \cdot L^{-1}$) was used as a substrate, whereas cells grew well on cellobiose and xylans (not shown).

Monitoring of cellulose and wheat straw degradation by analysis of the solid residue

R. albus 20 cells, grown in the presence of Sigmacell 20 or ^{13}C-labelled wheat straw, were harvested after 8, 16, 24, 48, 56, 72 and 96 h of growth. A similar experiment was carried out in parallel with *F. succinogenes* S85 for comparison. The pellet containing bacteria and the solid fibres, obtained after centrifugation, was freeze-dried and analysed further by ^{13}C-CP MAS NMR. The quantification of the ^{13}C signals of the crystalline and amorphous zones obtained on pellets of both cellulose and ^{13}C-labelled wheat straw was performed as described previously [7]. The CH_2 signals of polypropylene (PP at δ 43.8 p.p.m.) and polyethylene (PE at δ 32.8 p.p.m.) were used as internal reference signals. The spectra obtained showed that the signals of the crystalline and amorphous zones of cellulose, as well as the signals caused by hemicellulose, decreased at the same rate. This suggests that these substrates are degraded by *R. albus* 20 and *F. succinogenes* S85 at the same rate (Fig. 3). This result is similar to that previously observed on wheat straw with *F. succinogenes* S85 [7]. However, the comparison of the degree of degradation of both substrates after the same cultivation time showed a higher efficiency of *F. succinogenes* S85 (Fig. 3).

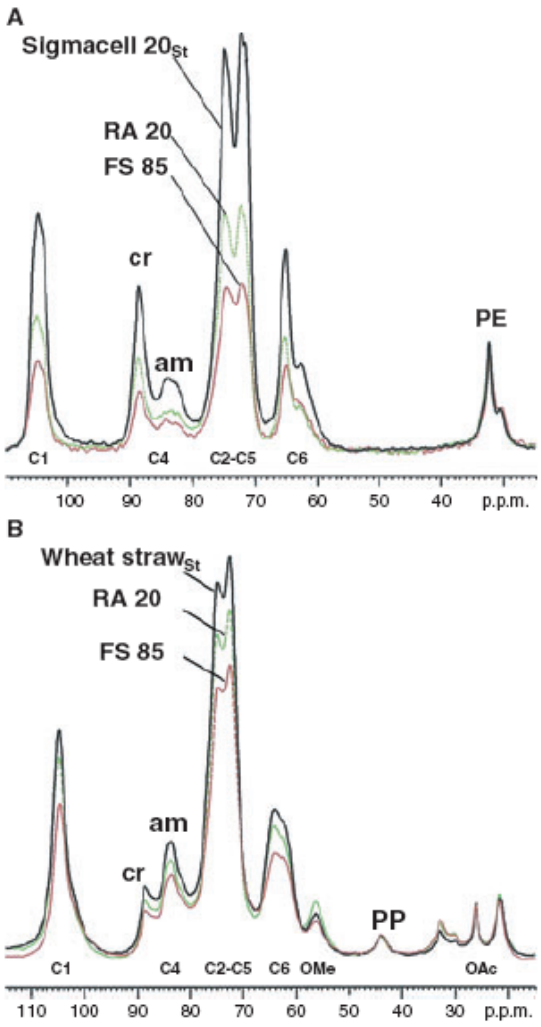

Fig. 3. ^{13}C-CP MAS NMR spectra of cellulose (A) and wheat straw (B) before and after the growth of *R. albus* 20 and *F. succinogenes* S85. St, Sigmacell 20 cellulose or wheat straw before bacterial inoculation (full black line); RA 20, after 4 days of *R. albus* 20 growth (green broken line); FS 85, after 4 days of *F. succinogenes* S85 growth (full red line). The experiments were performed at least in duplicate; representative spectra are shown here. am, signal of amorphous zone; C1–C6, assignment of carbon signals of cellulose (A) or cellulose and hemicellulose (B); cr, signal of crystalline zone; OAc, *O*-acetyl groups; OMe, *O*-methyl groups; PE, polyethylene standard; PP, polypropylene standard.

Monitoring of cellulose and wheat straw degradation by analysis of the culture medium

The degradation of cellulose and wheat straw by *R. albus* 20 was monitored by analysis of the components solubilized in samples of culture medium (taken at different time intervals after discarding the cell and straw pellet) by ^{1}H–^{1}H COSY and ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) NMR experiments. Table 1 shows the chemical shifts of the metabolites identified or searched for in the culture medium of *R. albus* 20 grown with cellulose and wheat straw.

Cellulose degradation

Figure 4A1 shows the anomeric region of the COSY spectrum and Fig. 4A2 shows the anomeric region of the heterocorrelated HSQC spectrum of the sample obtained after 4 days of culture of *R. albus* 20 with Sigmacell 20 cellulose. The cross-peaks of the nonreducing glucose unit CD_n of cellobiose (βGlc(1 → 4)Glc) and its reducing end glucose units CDα and CDβ, those of free αGlc and βGlc and the signal of an unknown metabolite X2 are found in Fig. 4A1. In the HSQC spectrum (Fig. 4A2), a characteristic chemical shift caused by the H1/C1 cross-peak signal at δ 5.46/94.52 suggests the presence of a derivative of Glc1*P* (marked *). However,

Table 1. Chemical shifts of the metabolites present or searched for in the culture medium of *R. albus* 20. Chemical shifts were determined in samples at 27 °C after pH correction to pH 7.4, or were from [7]. The H1 signal of 1-*O*-methyl-β-D-xylopyranose was taken as standard. αAraf^{Xyl}, α-arabinofuranose linked to O2, O3 or O2, O3 of xylose unit; Ara*p*, arabinopyranose; CB, cellobiose; CD, cellodextrin; αGalf^{Man}, α-galactofuranose in galactomannan or arabinogalactan; Glc, glucose; GlcA, glucuronic acid; $GlcA^{Xyl}$, α-glucuronic acid linked to O2 of xylose; Glc6*P*, glucose 6-phosphate; int, internal; Malt, maltose; Malt-1*P*, maltose phosphate; MD, maltodextrin; nr, nonreducing end; 1-O-Me-Xyl*p*, 1-*O*-methyl-β-D-xylopyranose used as standard; nd, not determined; red, reducing end; term, terminal; X2, unidentified derivative of glucose; Xyl, xylose; Xyl^{GlcA}, xylose unit substituted at O2 by α-glucuronic acid.

	Chemical shift δ (p.p.m.)				Chemical shift δ (p.p.m.)		
Residue	H1	C1	H2	Residue	H1	C1	H2
αGlc	5.24	92.93	3.54	CD_{term}	4.51	102.35	3.32
βGlc	4.65	96.75	3.24	αAra*p*	4.53	97.60	3.52
αGlc6*P*	5.24	93.07	3.58	βAra*p*	5.25	93.41	3.82
βGlc6*P*	4.65	96.92	3.28	αXyl	5.20	93.32	3.53
Glc1*P*	5.46	94.16	3.52	βXyl	4.59	97.64	3.23
Malt-1P_{nr}	5.43	100.41	3.58	Xyl_{int}	4.47	102.6	3.27
Malt-1P_{red}	5.46	94.28	3.52	βXyl_{red}	4.60	97.24	3.26
MD_{term}	5.41	100.59	3.59	αXyl_{red}	5.20	92.79	3.56
MD_{int}	5.41	100.41–100.37	3.63	Xyl^{GlcA}	4.63	102.4	3.43
αMD_{red}	5.24	92.74	3.58	$GlcA^{Xyl}$	5.32	98.30	3.58
βMD_{red}	4.66	96.60	3.28	αAraf^{Xyl}	5.3–5.1	110–107	4.1–4.0
CB_{nr}	4.52	103.31	3.32	αGalf^{Man}	5.10	108.2	nd
αCB_{red}	5.23	92.68	3.59	1-OMe-Xyl*p*	4.33	104.79	3.26
βCB_{red}	4.67	96.61	3.30	X2	4.63	101.1	3.38
CD_{int}[b]	4.53	102.18	3.36				

its broad signal at δ 5.46 in the ^{1}H NMR spectrum did not give any cross-peak in the COSY spectrum because of the very low $^3J_{H1,H2}$ and $^3J_{H1,31P}$ coupling constants. An aglycon part of the molecule may be the reason for this coupling constant change.

Figure 5A shows the concentration changes of the metabolites released during the growth of *R. albus* 20 with Sigmacell 20 cellulose. The NMR signal intensities of the identified metabolites were quantified in the ^{1}H NMR spectra at different times relative to those of the internal standard sodium 3-(trimethylsilyl) propionate (TSP-d_4). Glucose and cellodextrins accumulated with time in the culture medium. Glc1*P* was produced during the first 2 days, and then remained at a constant concentration. The concentration of X2 was rather low, and increased slowly with time. It should be noted that X2 was already present at time zero (0.16 mM), probably because of its presence in the bacterial culture used for inoculation. TLC analysis of the culture medium revealed that, in addition to a small quantity of cellobiose, the main component of cellodextrins is cellotriose (not shown).

Wheat straw degradation

Spots on the very complex *in situ* two-dimensional NMR spectra were identified by a comparative analysis with standards and literature data on the basis of their characteristic H1, H2 and C1 chemical shifts (Table 1).

Figure 4B1 shows part of a COSY spectrum of the culture medium obtained after 4 days of culture of *R. albus* 20 on wheat straw. The cross-peaks of free glucose, arabinose, X2 and xylose were detected. Signals of other metabolites could not be identified because of the low concentration and splitting of the signals by the $^1J_{1H,13C}$ coupling constant. However, they appeared in the HSQC spectrum.

The HSQC spectrum of the incubation medium obtained after 4 days of culture is shown in Fig. 4B2. The signals of free xylose, glucose and arabinose were identified. The signal intensity of X2 indicated that just traces were present. Characteristic signals of α-arabinofuranose ($Araf^{Xyl}$), which may be linked to both O2 and O3 or separately to O2 or O3 of xylose residues, suggested the presence of arabinoxylan oligosaccharides in the culture medium (Table 1, Fig. 4B2). The presence of glucuronoxylan oligosaccharides with substitution of the xylose units at O2 by 4-*O*-methyl glucuronic acid was suggested by the presence of $GlcA^{-Xyl}$ and Xyl^{-GlcA} signals (Table 1, Fig. 4B2).

Figure 5B shows the concentration changes of the metabolites released during the growth of *R. albus* 20 with wheat straw, and quantified as described above. Free xylose accumulated clearly in the culture medium with time (up to 1.4 mM after 4 days). Free glucose and arabinose also accumulated, but at a much lower level. The intensity of the cross-peak caused by the internal xylose units of xylooligosaccharide chains at δ

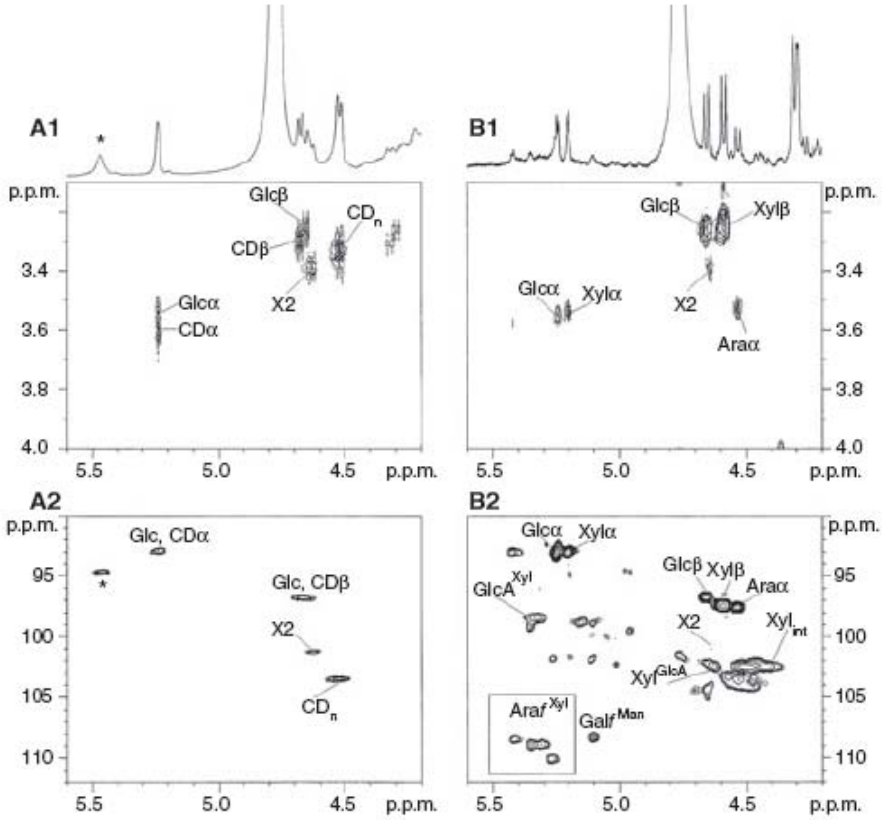

Fig. 4. Spectra of culture medium of *R. albus* 20 grown with cellulose (A) or wheat straw (B) after 4 days. (A1, B1) Anomeric part of COSY spectrum. (A2, B2) Anomeric part of HSQC spectrum. Ara, arabinose; $Araf^{Xyl}$, α-arabinofuranose linked to O2, O3 or O2,O3 of xylose; CDαβ, α- and β-glucose units of reducing end of cellobiose; CD_n, nonreducing end glucose of cellobiose or terminal and internal units of cellodextrins; $Galf^{Man}$, α-galactofuranose of galactomannans; Glc, glucose; $GlcA^{Xyl}$, α-glucuronic acid linked to O2 of xylose unit; X2, unknown derivative of glucose; Xyl, xylose; Xyl^{GlcA}, xylose unit substituted at O2 by αGlcA; Xyl_{int}, internal units of nonsubstituted xylooligosaccharides; *, glucose 1-phosphate derivative.

4.47/3.27 remained at trace level during the first 24 h and could not be detected at 96 h, suggesting their rapid utilization by cells or their degradation to free xylose. The low-intensity signals of galactomannan and glucuronoxylan oligosaccharides were present at the start of culture and did not increase with time. In addition, the intensity of the α-galactofuranose signal (αGal*f*) did not change during incubation. X2 was barely detectable during incubation.

Discussion

In this study, we analysed the action of the fibrolytic system of *R. albus* on the different components of cellulose and wheat straw used as substrates for growth by the bacterium, and compared the results with those previously observed with *F. succinogenes*. Although solid-state NMR has been used successfully previously to study the action of fibrolytic organisms on lignocellulose [8,9], the present study confirmed the value of combining both solid- and liquid-state NMR to monitor the action of cellulolytic bacteria on complex substrates, such as wheat straw. The first important result of this work was that ^{13}C-CP MAS NMR analysis did not show the preferential degradation of amorphous versus crystalline regions of cellulose in wheat straw or pure Sigmacell 20 cellulose. This suggests either the simultaneous degradation of the amorphous and crystalline parts of cellulose by the enzymes, or degradation at the surface, at a molecular scale, that cannot be detected by NMR. This result is similar to that obtained with *F. succinogenes* [7], and suggests that, for both cellulolytic strains, cellulases do not degrade the amorphous regions of cellulose more quickly in pure cellulose or wheat straw. In addition, the ^{13}C-CP MAS NMR results showed that cellulose and hemicellulose were degraded at the same rate in wheat straw. Again, the simultaneous degradation of cellulose and hemicellulose by the *R. albus* 20 enzymatic system, or degradation at the surface, can be proposed to explain these results.

The second important result of this study was the accumulation of soluble mono- and oligosaccharides in the medium of both cellulose and wheat straw cultures of *R. albus* 20, observed using two-dimensional NMR techniques. In the rumen ecosystem, these sugars can be used by other bacteria and thus participate in cross-feeding between cellulolytic and noncellulolytic species [10]. Glucose accumulated in significant amounts in the cellulose culture medium, and also to some extent in wheat straw cultures. It may be released from cellulose, cellodextrins or other glucans (xyloglucans, etc.) in the case of wheat straw hydrolysis. Its accumulation suggests that *R. albus* 20 does not use this sugar. Indeed, we determined that *R. albus* 20 was unable to

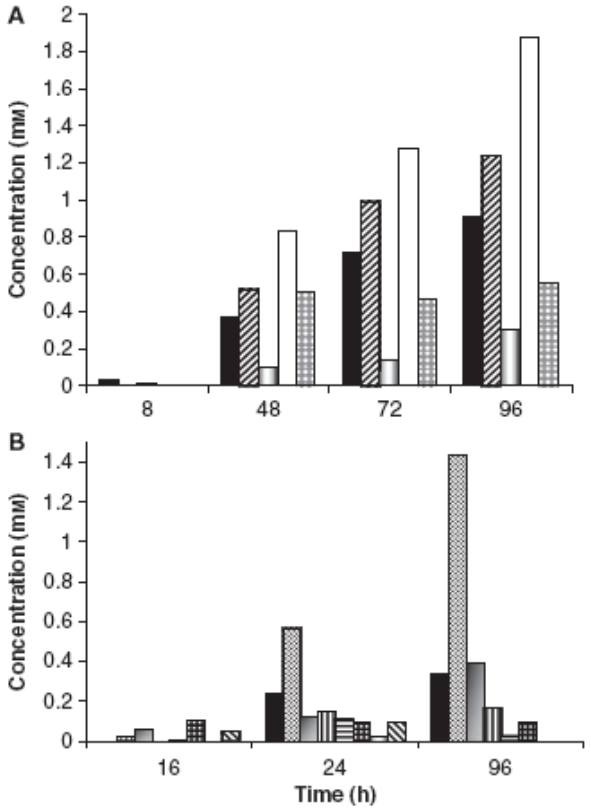

Fig. 5. Time dependence of concentration of metabolites released during the growth of *R. albus* 20 on Sigmacell 20 cellulose (A) or wheat straw (B). Values were determined from the signal intensities in the ^{1}H NMR spectra. At least two signal integrations were carried out. Standard deviations were usually less than 15%. Ara; αAra^{Xyl}; α and β CB_{red} or α and β cellodextrins (CD_{red}); CD_{int}; $\alpha Galf^{Man}$; α and β Glc; Glc-*IP*; Glc^{Xyl}; X2; Xyl; Xyl_{int}.

grow on glucose, probably because of a lack of a glucose transporter. Different strains of *R. albus* show different behaviour with regard to monosaccharide utilization [11]. Although strain B199 of *R. albus* is able to use glucose and cellobiose for growth, it clearly shows the preferential utilization of cellobiose over glucose, and this preference is related to the repression of the glucose uptake system in cellobiose-grown cells [12]. Our results showed that cellodextrins accumulated in large amounts in the cellulose culture medium, mainly as cellotriose. This accumulation may be the result of either a low rate of uptake of cellotriose compared with the other cellodextrins released from cellulose hydrolysis, or an efflux of cellotriose from the cells, as proposed previously for *R. albus* [13]. In addition, a compound incompletely characterized and named X2 accumulated in the culture medium, although at a low concentration. It was present at the start of culture, and originated from the culture inoculum. This compound appeared to be associated with cellulose degradation as it was barely detectable in wheat straw cultures.

In the wheat straw cultures, free xylose also accumulated to a significant extent (1.4 mM). As for glucose, the pentose utilization ability also appears to be variable between strains of *R. albus* [11]. Again, strain B199 was able to use xylose and arabinose, but preferentially utilized the products of cellulose degradation, and, in particular, cellobiose rather than hemicellulose digestion [14]. Indeed, we also determined that *R. albus* 20 was unable to grow on culture medium with xylose as substrate, indicating that this strain was unable to use xylose, either because of a lack of a transporter or of the xylose isomerase or xylulokinase, as shown previously for *F. succinogenes* [15]. However, *R. albus* 20 grows on xylans, and thus should use xylodextrins. This agrees with the observation that the concentrations of xylooligosaccharides (Xyl_{int}) were very low in the culture medium (Fig. 5B). Similarly, the concentrations of substituted xylooligosaccharides were quite low, and did not increase significantly with time, suggesting that the esterases of *R. albus* 20 are very active. No acetylated xylan oligosaccharides (at both the O2 and O3 positions of xylose) were detected, although it is known that stem wheat straw is highly acetylated [16]. This suggests, as for *F. succinogenes*, a high activity of acetylesterase. Free arabinose accumulated in the culture medium with time, showing the activity of the arabinofuranosidase; this result is consistent with the fact that *R. albus* 20 does not use arabinose for growth, and also with the small amounts of arabinose bound to xylooligosaccharides (Ara*f*). Galactose in galactomannans was also only found at a low concentration. It should be noted that, in the wheat straw culture medium, glucose was found in smaller amounts than in the cellulose culture medium, and cellodextrins were not detected at all. This suggests that cellulose is degraded at a lower rate in wheat straw, probably because of cellulase access limitation by hemicellulose, and thus cellodextrins are used by *R. albus* cells as soon as they are produced, as shown previously for *F. succinogenes* [7].

^{13}C-CP MAS NMR analysis of cellulose and wheat straw degradation by *R. albus* 20 and *F. succinogenes* S85 showed that *F. succinogenes* degraded both the homopolymer and the straw at a much higher rate (Fig. 3). Previous studies have shown a dominance of *F. succinogenes* S85 over the other fibrolytic rumen species *R. flavefaciens*, *Butyrivibrio fibrisolvens* and strain 7 of *R. albus* in determining the extent of lucerne cell wall degradation in co-cultures [5]. However, a more rapid degradation of barley straw by *R. flavefaciens* relative to *F. succinogenes* has also been observed [17]. Our results

also showed a clear dissimilarity in the behaviour of *R. albus* 20 and *F. succinogenes* S85 cultures on wheat straw, indicating differences in hemicellulose degradation and metabolism. First, although, in both cases, xylose accumulated in the culture medium, its concentration was much lower in *R. albus* than in *F. succinogenes* cultures, where its concentration reached about 6 mM [7]. Second, the concentration of arabinose was also higher in *F. succinogenes* cultures (2.5 mM) [7]. These results may be explained by a greater efficiency of *F. succinogenes* in hemicellulose degradation and, in particular, its arabinofuranosidase and xylanases. In addition, *F. succinogenes* accumulated xylooligosaccharides in much larger amounts, because this bacterium is unable to use xylooligosaccharides. As both polysaccharide degradation ability and sugar metabolism are important in the fibre degradation process by the two fibrolytic bacteria, it would be interesting to analyse by NMR the degradation of solid substrates by co-culture of the two species alone and in combination with non-cellulotytic species that are able to use the released sugars. This should aid in the understanding of the mechanisms which cause the predominance of certain fibrolytic species in the ecosystem.

Materials and methods

Bacterial growth

R. albus 20 (ATCC 27211) and *F. succinogenes* S85 (ATCC 19169) were grown in triplicate at 38 °C on 10 mL of mineral medium [7,18] with cellobiose (0.3% w/v), Sigmacell 20 cellulose (10 $g{\cdot}L^{-1}$) and unlabelled or ^{13}C-labelled wheat straw (10 $mg{\cdot}mL^{-1}$, 10% ^{13}C total enrichment). For the determination of sugar utilization, *R. albus* 20 was also grown on mineral medium with xylans (10 $g{\cdot}L^{-1}$), xylose, arabinose or glucose (3 $g{\cdot}L^{-1}$) as substrate. Wheat straw was ground into fine particles (< 500 μm) using a blender before incubation. Cell cultures (in triplicate) on cellulose or wheat straw were harvested after 8, 16, 24, 48, 56, 72 and 96 h of growth. The extracellular medium was separated from the cells and solid substrate by centrifugation (15 min at 20 000 *g*) before analysis. Supernatants and pellets were freeze-dried and analysed by two-dimensional liquid-state NMR and solid-state ^{13}C-CP MAS NMR spectroscopy, respectively.

NMR experiments

Solid-state NMR

For solid-state measurements, 50 mg of freeze-dried Sigmacell 20 cellulose or ^{13}C-enriched wheat straw (with or without cells) was mixed with 50 μL of water and 10 mg of PE or PP, respectively. The 4 mm ZrO_2 rotors were filled with these mixtures. High-resolution solid-state ^{13}C-CP MAS NMR spectra were measured on a Bruker Avance DSX spectrometer (Bruker Biospin SA, Wissenbourg, France) operating at 75.46 MHz in a commercial Bruker double-bearing probe. The acquisition of 2000 scans for each sample was performed at 10 kHz at room temperature using a variable-amplitude cross-polarization sequence and a standard pulse program of the Bruker library, with a 3.3 μs proton 90° pulse, 1 ms contact time and 5 s relaxation delay. Chemical shifts were referenced to the external standard glycine (δ 176.03 p.p.m.).

Liquid-state NMR

After pellet separation, the pH of the cell-free supernatants was corrected to pH 7.40 and the supernatants were freeze-dried twice with D_2O. Samples were further dissolved in a mixture of 470 μL of 99.98% D_2O, 20 μL of 10 mM TSP-d_4 (δ 0.0) and 10 μL of 50 mM 1-*O*-methyl-β-D-xylopyranose (δ 4.331/104.79) used as standards. Samples were subjected to liquid-state NMR measurements on a Bruker Avance DSX spectrometer operating at 300 and 500 MHz in 5 mm TXI inverse probes (^1H, ^{13}C, ^{15}N) with z-gradients at 27 °C. The following techniques were used for the assignment of NMR signals: two-dimensional gradient-enhanced proton-homonuclear shift correlation spectroscopy; one-dimensional transient gradient-enhanced nuclear Overhauser effect spectroscopy [19]; one-dimensional gradient-enhanced total correlation spectroscopy; gradient-enhanced heteronuclear single quantum coherence spectroscopy; and heteronuclear single quantum coherence distortionless enhanced polarization transfer spectroscopy [20]. To enhance the sensibility, ^1H–^{13}C correlated experiments were performed on supernatants obtained from incubations with ^{13}C-enriched straw.

To maintain the same quantity of salts, samples of standards were dissolved in the buffer used for incubation and, after pH correction to pH 7.4, were freeze-dried and dissolved in D_2O.

For both cellulose and wheat straw incubations, spectra at T0 (immediately after the addition of bacteria to the incubation medium) were measured, and the concentrations of the metabolites found were subtracted from those observed at the given times. The concentrations of the metabolites were calculated from the quantification of the signals in the ^1H NMR spectra relative to those of the internal standard TSP-d_4.

TLC

TLC was carried out as described in [21] using a mixture of glucose, cellobiose or phosphorylated sugars (from Sigma-Aldrich, Saint-Quentin Fallavier, France, 3 $g{\cdot}L^{-1}$) as standard.

Metabolite assays

Acetate, formate, ethanol and lactate were quantified from one-dimensional ^{1}H NMR spectra using TSP-d_4 as an internal reference: 0.5 mL of extracellular medium obtained after centrifugation of the cultures (15 min at 20 000 ***g***) was added to 50 mM TSP-d_4 and analysed by ^{1}H NMR (Bruker Avance DSX at 500 MHz). Peak areas were integrated and the metabolite concentration was calculated relative to TSP-d_4. Lactate, acetate and formate were also assayed using enzymatic kits (Roche Diagnostics, Meylan, France). All the assays were performed at least in duplicate.

Production of ^{13}C-enriched wheat straw

Durum wheat (cv. Ardente) was cultivated in airtight chambers with CO_2 (10% of $^{13}CO_2$), as described previously [7]. Plants were harvested after 104 days of culture and dried. The stems were used. The straw composition was the same as that described previously [7].

Chemicals

TSP-d_4 was purchased from Eurisotop (Saint-Aubin, France). 1-*O*-Methyl-β-D-xylopyranose, PP, PE and all other chemicals were purchased from Sigma-Aldrich.

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